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# INHIBITION OF HUMAN CYTOMEGALOVIRUS PROTEASE $N_o$ WITH MONOCYCLIC β-LACTAMS

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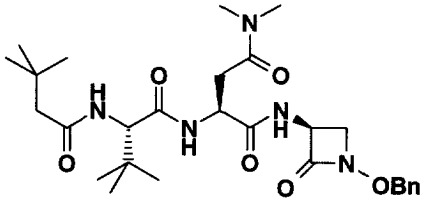
**Abstract:** Monocyclic β-lactams have been identified as potent and selective inhibitors of the human cytomegalovirus protease (HCMV) $N_o$. Two series of these inhibitors are described, a peptidyl series of compounds and non-peptidic molecules featuring lower molecular weights. The SAR work that lead to the discovery of these inhibitors, together with their synthesis is also disclosed. 

Human cytomegalovirus, like all herpesviruses, encodes a unique protease that is necessary for viral replication.[1] Recent determination of the X-ray structure of the HCMV N-terminal protease domain ($N_o$) of the UL80 gene product has revealed that this enzyme belongs to a novel class of serine protease.[2] The active site is composed of a triad consisting of His63, His157 and Ser132. Since their discovery, herpesvirus proteases have become attractive molecular targets for the design of novel antiviral agents.[3]

We recently reported a series of substrate-based activated carbonyl inhibitors of HCMV protease.[4] In that study we disclosed the peptidyl β-lactam **1**, which inhibited HCMV protease $N_o$ with an $IC_{50}$ of 33 μM. We now report SAR results that have resulted in the identification of more potent and non-peptidic series of monocyclic β-lactam inhibitors of the HCMV protease.




**1**

Encouraged by the fact that we could inhibit the HCMV protease $N_o$ with β-lactams, we pursued our SAR investigations by attaching the peptidic pharmacophore onto the C-4 position of the β-lactam ring. For reasons of chemical simplicity this moiety was attached through an ether linkage. In the substrate based series,[4] we had found that the (*S*)-*N,N*-dimethylasparagine and the *t*-butylacetyl residues could be replaced by leucine and *t*-butyloxycarbonyl groups, respectively. These replacements were chosen for the present study so as to facilitate the preparation of inhibitors. The β-lactam derivative **2** was therefore prepared (Scheme 1) but was inactive ($IC_{50}$ > 300 μM, Table 1). This lack of inhibitory activity could be attributed to a poor fit of **2** into the active site of the enzyme and/or to a lack of electrophilicity of the β-lactam carbonyl. Since it had

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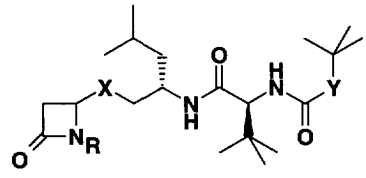
already been shown by Merck researchers that N-carbonylamino derived β-lactams are potent inhibitors of human leukocyte elastase[5] we undertook to incorporate such functionalities into **2**. Treatment of **2** with benzylisocyanate afforded **3**, which turned out to be quite active with an $IC_{50}$ of 1.3 μM. This result prompted us to prepare the (*R*) and (*S*) phenylethyl derivatives **4** and **5**. The introduction of the (*R*) stereocenter improved activity by fourfold relative to compound **3**.[6] Further SAR studies indicated that the oxygen atom at C-4 could be replaced by sulfur (cf. **3** and **6**, note that compound **3** is a 1:1 mixture of isomers). Replacement of the Boc group on **6** by *t*-butylacetyl and incorporation of the *R*-1-phenylpropionyl urea gave the most potent inhibitor described in this paper (**7**) with an $IC_{50}$ of 0.07 μM. This compound represents one of the most active inhibitors of HCMV protease reported to date.

**Table 1: Inhibitory activity of substrate-based β-lactam inhibitors against HCMV protease $N_o$[7].**



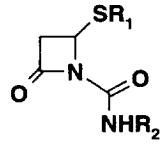
| Compound | R | X | Y | $IC_{50}$ μM[a] (SD)[b] |
|---|---|---|---|---|
| 2 | H | O | O | >300 |
| 3 | CONH–Ph | O | O | 1.3 (0.1) |
| 4 | CONH–Ph | O | O | 0.3 (0.04) |
| 5 | CONH–Ph | O | O | 1.4 (0.4) |
| 6 | CONH–Ph | S | O | 0.4 (0.1) |
| 7 | CONH–Ph | S | $CH_2$ | 0.07 (0.01) |

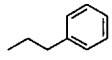
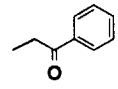
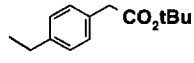
[a] Compounds **2**, **3** and **7** were assayed as 1:1 mixtures of diastereoisomers at C-4 whereas the reported $IC_{50}$ values of compounds **4-6** are those of the most active diastereoisomer.
[b] Average of three determinations.

Attempts to further increase potency in this series of compounds resulted in incremental improvements only. We therefore focused our efforts on reducing the peptidic character and molecular weight of these inhibitors. Replacement of the peptidic appendage at C-4 by a phenyl group (Table 2, compound **8**) resulted in a 1.9 μM inhibitor. Extension of this side-chain by one or two methylene units had a deleterious effect on the inhibitory activity by as much as threefold (cf. **8** and **10**). The incorporation of a picolyl residue was then investigated, the three possible isomers were made (**11**, **12**, and **13**) and only minor difference in inhibitory

activity was observed. Several other moieties were also investigated at this position and nothing substantially better was identified (e.g. **14** and **15**). Some modifications to the $R_2$ substituent were then surveyed.

**Table 2: Inhibitory activity of 4-thiosubstituted β-lactams against HCMV protease $N_o$[7].**

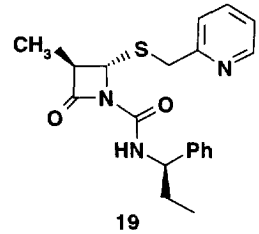



| Compound | $R_1$ | $R_2$ | $IC_{50}$ μM[a] (SD)[b] |
|---|---|---|---|
| 8 |  |  | 1.9 (0.2) |
| 9 |  |  | 2.4 (1.0) |
| 10 |  |  | 6.5 (1.5) |
| 11 |  |  | 2.1 (0.1) |
| 12 |  |  | 2.0 (0.6) |
| 13 |  |  | 2.5 (0.6) |
| 14 |  |  | 1.0 (0.2) |
| 15 |  |  | 75 (9) |
| 16 |  |  | 1.2 (0.3) |
| 17 |  | $CH_2CH_2OBn$ | 6.4* (0.1) |
| 18 |  |  | 3.6 (1.8) |

[a] The reported $IC_{50}$ values are those of the most active diastereoisomer.
[b] Average of three determinations.
*Tested as a racemic mixture.

Replacement of the phenylethyl group by a phenylpropionyl moiety (**13** and **16**) resulted in a twofold improvement in enzymatic inhibition. Incorporation of more polar groups at this position (**17** and **18**) gave

small losses in potency. We next turned our attention to the C-3 position of the β-lactam ring. Thus, the 3*S*-methyl analog **19** was made *via* a stereospecific synthesis from D-aspartic acid.[5] We were pleased to observe that this modification resulted in a threefold increase in potency ($IC_{50}$ = 0.35 μM) compared with **16**. The stereospecific introduction of the C-3 methyl group also allowed us to assess the relative configuration of the C-4 substitutent by NMR analysis. The coupling constant of 3.2 Hz between H-3 and H-4 is typical of a *trans* relationship between the two substitutents. One could therefore postulate that the configuration at the C-4 carbon of the most active isomers of the β-lactams disclosed herein is *S*, as is the case for **19**.




**19**

Since similar β-lactams had already been reported to be potent inhibitors of Elastase[5], we assessed the selectivity profile our compounds. Compounds **3** to **19** were evaluated for activity against three serine proteases; human leukocyte elastase (HLE), porcine pancreatic elastase (PPE), α-chymotrypsin (α-Chym), and cathepsin-B (Cat-B), a cysteine protease. As shown in Table 3, the overall specificity profile of these β-lactams derivatives turned out to be excellent with the notable exception of compound **19**. It is clear that the incorporation of a methyl group at the C-3 position seriously altered the selectivity profile (cf. **16** and **19**). This result is consistent with previous reports of monobactam HLE inhibitors[5] which showed that the presence of an alkyl group at position 3 was essential for activity. Preliminary studies on the mode of inhibition of HCMV $N_0$ by these molecules indicate the reversible and competitive formation of an acyl-enzyme intermediate. A detailed account of these studies will be reported in due course.[8] The present inhibitors were also evaluated for their ability to prevent viral replication in a plaque reduction assay.[7] Two compounds were found to exhibit acceptable antiviral activity, compounds **11** and **16** with $EC_{50}$ values of 60 μM and 73 μM, respectively. No cytotoxicity was observed at these concentrations ($TC_{50} \approx 200$ μM).

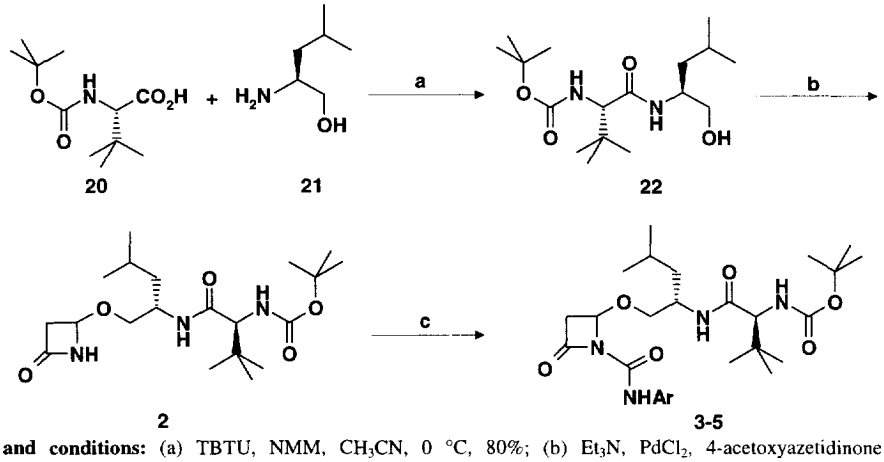
The general synthetic protocol for the preparation of inhibitors **2** to **5** is outlined in Scheme 1. Boc-protected *t*-butylglycine **20** was coupled with *S*-leucinol (**21**) using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3 tetramethyluronium tetrafluoroborate (TBTU) (1.02 equiv) and *N*-methylmorpholine (1.10 equiv) in acetonitrile at 0 °C to give **22** in 80% yield. The β-lactam **2** was then obtained in 46% yield by treating 4-acetoxyazetidinone (**23**) with alcohol **22** in the presence of $PdCl_2$ (0.20 equiv) and triethylamine (1.70 equiv) in a mixture of dichloromethane:toluene:DMF (5:15:3) at room temperature. Condensation of **2** with the appropriated isocyanate in the presence of triethylamine (1.30 equiv) and a catalytic amount of DMAP in dichloromethane at room temperature afforded the desired β-lactams **3** to **5** in 46% to 67% yield as mixtures

**Table 3: $IC_{50}$ (μM) of β-lactams 3-19 against various proteases.**

| Compound | HLE | PPE | α-Chym | Cat-B |
|---|---|---|---|---|
| **3** | >75 | >75 | 8.3 | >75 |
| **4** | >75 | >75 | >75 | >75 |
| **5** | >75 | >75 | >75 | >75 |
| **6** | >75 | >75 | 0.8 | >75 |
| **7** | >75 | >75 | >75 | >75 |
| **8** | >75 | 6.3 | >75 | >75 |
| **9** | >75 | >75 | >75 | >75 |
| **10** | >75 | >75 | >75 | >75 |
| **11** | >75 | 21 | >75 | >75 |
| **12** | >75 | 65 | >75 | >75 |
| **13** | >75 | >75 | >75 | >75 |
| **14** | >75 | >75 | >75 | >75 |
| **15** | >75 | >75 | >75 | >75 |
| **16** | >75 | 23 | >75 | >75 |
| **17** | >75 | 40 | >75 | >75 |
| **18** | >75 | >75 | >75 | >75 |
| **19** | <0.15 | 0.73 | 1.7 | >75 |

of diastereoisomers. The 4-thio-β-lactam derivatives **6** to **18** were prepared according to a slightly modified protocol (Scheme2). The appropriate alcohols were treated with thiolacetic acid under Mitsunobu conditions

**Scheme 1**




20 21 22 2 3-5

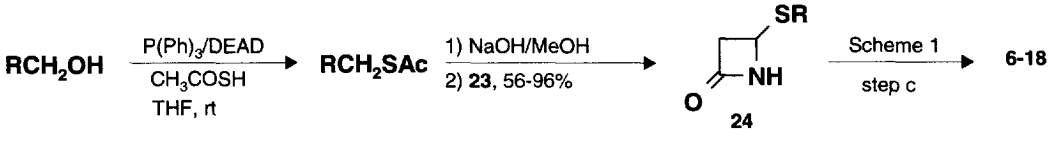
**Reagents and conditions:** (a) TBTU, NMM, $CH_3CN$, 0 °C, 80%; (b) $Et_3N$, $PdCl_2$, 4-acetoxyazetidinone (**23**), $PhCH_3$:$CH_2Cl_2$:DMF (5:15:3), rt, 46%; (c) ArNCO, $Et_3N$, DMAP (cat), $CH_2Cl_2$, rt, 46-67%.

to give the corresponding thioacetate intermediates. Saponification with aqueous sodium hydroxide in methanol followed by the addition of **23** afforded β-lactam intermediates **24** in 56% to 96% yield. The desired compounds **6-18** were then obtained by treating **24** with the appropriated isocyanates employing the reaction conditions described in Scheme 1. The C-4 diastereoisomers were separated by silica gel column chromatography. β-lactam **19** was prepared by employing the reaction conditions described in Scheme 2

using (3*S*,4*S*)-3-methyl-4-acetoxyazetidinone (prepared from D-aspartic acid according to the published protocol[5]) in place of 4-acetoxyazetidinone **23**.

In conclusion, monobactam-based inhibitors of the HCMV protease $N_o$ have been identified. The peptidic series exhibited good *in vitro* potency and was selective when tested for inhibitory activity using a number of serine and cystine proteases. Moreover, some of the compounds in the smaller series exhibited activity in the viral replication assay.

**Scheme 2**

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6. Reported inhibitory activities of compounds **4-6**, and **8-18** were obtained from the most active diastereoisomers which were isolated by silica gel chromatography. Compounds **2**, **3** and **7** were assayed as mixtures of diastereoisomers.
7. All compounds were assayed according to the protocols described in ref. 4.
8. Bonneau, P. Manuscript in preparation.